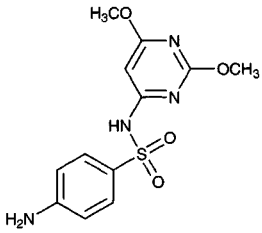
Sulfadimethoxine



Molecular formula: $C_{12}H_{14}N_4O_4S$
Molecular weight: 310.33
CAS Registry No.: 122-11-2
Merck Index: 9073
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 3 mL MeOH and 3 mL 50 mM pH 3.4 oxalate buffer. 1 mL Plasma + 1 mL 50 mM pH 3.4 oxalate buffer + 50 μL MeOH:water 50:50, mix, add to the SPE cartridge. Wash with 3 mL 50 mM pH 3.4 oxalate buffer, 1 mL MeOH:water 20:80, and 2 mL hexane:ether 80:20. Elute with two 1 mL portions of MeOH:25% ammonia solution 99:1. Evaporate the eluate to dryness under a gentle stream of nitrogen at 30°. Dissolve the residue in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.9 5 μm Symmetry C18 (Waters)
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: MeCN:MeOH:water 25:10:65 containing 1% triethylamine, adjust to p 5.6 with phosphoric acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Internal standard: sulfadimethoxine

OTHER SUBSTANCES

Extracted: pyrimethamine, sulfadoxine
Simultaneous: acetaminophen, 4-chlorophenylbiguanide, cycloguanil, proguanil, quinine, sulfadiazine

KEY WORDS

plasma; SPE; sulfadimethoxine is IS

REFERENCE

Astier,H.; Renard,C.; Cheminel,V.; Soares,O.; Mounier,C.; Peyron,F.; Chaulet,J.F. Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction, *J.Chromatogr.B*, **1997**, *698*, 217–223.

SAMPLE

Matrix: blood, milk
Sample preparation: 1 mL Serum or milk + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 10 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL p-aminobenzoic acid in 0.01% trichloroacetic acid, centrifuge at 1000 g for 10 min. Remove a 500 μL aliquot of the clear layer and add it to 100 μL 1 mg/mL fluorescamine in acetone (prepared fresh each day), mix for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm Nova-Pak C18
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 18.5
Internal standard: p-aminobenzoic acid (5.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, sulfathiazole

KEY WORDS
cow; serum; derivatization

REFERENCE
Tsai,C.-E.; Kondo,F. Liquid chromatographic determination of fluorescent derivatives of six sulfonamides in bovine serum and milk, *J.AOAC Int.*, **1995**, *78*, 674–678.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 200 μL Plasma + 90 μL 24% trichloroacetic acid in MeOH + 10 μL 10 μg/mL sulfamethoxazole in MeOH, vortex for 30 s, centrifuge at 14000 g for 5 min, inject a 50 μL aliquot of the supernatant. Muscle. Homogenize 1 g muscle in 1.5 mL MeOH:buffer 20:80, add 50 μL 10 μg/mL sulfamethoxazole in MeOH, mix thoroughly for 1 min, centrifuge at 14000 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm ODS-Hypersil
Column: 150 × 4.6 3 μm ODS-Hypersil C18
Mobile phase: MeCN:buffer:triethylamine 20:80:0.02 (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 14
Internal standard: sulfamethoxazole (8)
Limit of detection: 30 ng/g (muscle), 15 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: ormetoprim

KEY WORDS
plasma; muscle; fish; salmon

REFERENCE
Samuelsen,O.B. Simultaneous determination of ormethoprim and sulphadimethoxine in plasma and muscle of Atlantic salmon *(Salmo salar)*, *J.Chromatogr.B*, **1994**, *660*, 412–417.

SAMPLE
Matrix: blood, tissue
Sample preparation: 1 mL Serum or homogenized tissue + 4 mL MeCN, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL water, mix vigorously, add 1 mL MeCN, centrifuge at 1000 g for 15 min. Remove the upper layer and evaporate it to dryness, reconstitute the residue in 1 mL 10 ng/mL sulfadiazine in 0.01% trichloroacetic acid, shake, add 100 μL hexane, shake, centrifuge at 1000 g for 15 min. Remove a 500 μL aliquot of the clear aqueous layer and add it to 100 μL 1 mg/mL fluorescamine in MeCN (freshly prepared), shake by hand for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Nova-Pack C18

Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 475

CHROMATOGRAM
Retention time: 18.2
Internal standard: sulfadiazine (7.1)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: sulfamethoxazole, sulfamonomethoxine, sulfamethazine

KEY WORDS
serum; pig; derivatization; kidney; muscle; liver

REFERENCE
Tsai,C.-E.; Kondo,F. A sensitive high-performance liquid chromatographic method for detecting sulfonamide residues in swine serum and tissues after fluorescamine derivatization, *J.Liq.Chromatogr.*, **1995**, *18*, 965–976.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.735

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water

pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 5.0
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: eggs, tissue
Sample preparation: Homogenize (Nippon Seiki AM-1) 5 g tissue or eggs with 25 mL MeCN: water 90:10 and 20 mL hexane, centrifuge at 2100 g for 10 min, filter (Toyo Roshi No. 2 paper) the supernatant, repeat the extraction twice more. Leave the combined filtrate until phase separation is complete, dry the MeCN layer over anhydrous sodium sulfate, filter, add to the alumina column, wash with 30 mL MeCN, elute with 20 mL MeCN:water 90:10. Evaporate the eluate to dryness and reconstitute the residue with 1 mL mobile phase, inject a 20 μL aliquot. (Prepare the alumina column by adding 6 g 60-200 μm 90 active basic (activity I) aluminum oxide (Merck) to a 300 × 15 column, wash with 30 mL MeCN:water 90:10, wash with 30 mL MeCN.)

HPLC VARIABLES

Guard column: 4 × 4 7 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 25:75
Flow rate: 1

Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 12.4
Limit of detection: 10 ppb

OTHER SUBSTANCES
Extracted: metabolites, N-acetylsulfadimethoxine, N-acetylsulfamonomethoxine, sulfamonomethoxine

KEY WORDS
pig; cow; chicken; muscle; SPE

REFERENCE
Furusawa,N.; Mukai,T. Simultaneous high-performance liquid chromatographic determination of residual sulphamonomethoxine, sulphadimethoxine and their N4-acetyl metabolites in foods of animal origin, *J.Chromatogr.A*, **1994**, *677*, 81–85.

SAMPLE
Matrix: formulations
Sample preparation: Powder 20 tablets, dissolve a portion of the powder in water such that the concentration of penicillin V potassium is 0.6 mg/mL, mix well, filter. Mix 20 mL filtrate with 15 mL 0.4 mg/mL sulfadimethoxine in MeCN:water 50:50, dilute to 100 mL with water, mix well, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 mm long 30-50 μm Whatman Co:Pell ODS
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 21:4:75
Flow rate: 1-1.5
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Internal standard: sulfadimethoxine

OTHER SUBSTANCES
Simultaneous: penicillin V

KEY WORDS
tablets; collaborative study; sulfamdimethoxine is IS

REFERENCE
Mopper,B. Liquid chromatographic determination of penicillin V potassium in tablets: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 883–884.

SAMPLE
Matrix: milk
Sample preparation: Mix 5 mL milk with 20 μL 12 M HCl, sonicate, add 25 mL ethyl acetate, extract using a rotary shaker (REAX 2, Heidolph) for 10 min. Centrifuge at 1500 g for 5 min, evaporate 20 mL of the ethyl acetate extract to dryness, dissolve the residue in 10 mL 1 HCl. Wash the aqueous phase with 10 mL hexane, adjust to pH 5.5 with 900 μL 10 M NaOH and 5 mL 1 M pH 6.0 KH_2PO_4, extract with two 10 mL portions of dichloromethane. Evaporate the organic layer to dryness, dissolve the residue in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Novapak C18 (Waters)
Mobile phase: MeCN:10 mM pH 6.6 ammonium acetate 10:90
Flow rate: 1

Injection volume: 50
Detector: UV 271

CHROMATOGRAM
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Also analyzed: sulfadimidine (UV 265), sulfadoxine, sulfamethoxypyridazine (UV 265)

KEY WORDS
cow; milk

REFERENCE
Roudaut,B.; Moretain,J.P. Sulphonamide residues in milk of dairy cows following intravenous injection, *Food Addit.Contam.*, **1990**, *7*, 527–533.

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 2 g C18 material + 10 μL MeOH + 10 μL 12.5 μg/mL sulfamerazine in MeOH, let stand for 1 min, grind with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane, elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 100 μL MeOH and 400 μL 17 mM orthophosphoric acid, sonicate for 5-10 min, centrifuge at 13600 g for 5 min, filter supernatant (0.45 μm), inject a 20 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 75 × 4 3 μm Supelcosil LC-18
Mobile phase: MeCN:17 mM orthophosphoric acid 10:90
Column temperature: 45
Flow rate: 1 for 5 min then 2 for remainder of run
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 15.5
Internal standard: sulfamerazine (3)
Limit of detection: 62.5 ng/mL

OTHER SUBSTANCES
Simultaneous: sulfamethoxazole, sulfanilamide, sulfathiazole, sulfadiazine, sulfamethazine, sulfisoxazole

REFERENCE
Long,A.R.; Short,C.R.; Barker,S.A. Method for the isolation and liquid chromatographic determination of eight sulfonamides in milk, *J.Chromatogr.*, **1990**, *502*, 87–94.

SAMPLE
Matrix: milk
Sample preparation: Wash filter paper with 5 mL chloroform:acetone 2:1, discard filtrate. Extract 10 mL milk with 50 mL chloroform:acetone 2:1 by shaking for 4 min with periodic venting, let stand for 5 min, repeat extraction with 25 mL chloroform:acetone 2:1. Filter the organic layers, wash the filter paper with two 5 mL portions of chloroform:acetone 2:1. Evaporate the filtrate just to dryness under reduced pressure at 32 ± 2°, reconstitute the residue with 1 mL 13.6 g/L KH_2PO_4, vortex for 1 min, add 5 mL hexane, vortex for 1 min, let stand for 2 min, vortex for 1 min, let stand for at least 15 min, filter (2 μm) the aqueous layer, inject a 100 μL aliquot of the filtrate

HPLC VARIABLES
Guard column: 20 mm long Supelco guard column
Column: 250 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:13.6 g/L KH_2PO_4 30:70
Column temperature: 35
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 15.0
Limit of detection: 0.7 ppb
Limit of quantitation: 1.6 ppb

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfamethazine, sulfaquinoxaline

KEY WORDS
cow

REFERENCE
Smedley,M.D.; Weber,J.D. Liquid chromatographic determination of multiple sulfonamide residues in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 875–879.

SAMPLE
Matrix: milk
Sample preparation: 5 mL Milk + 100 μL concentrated HCl, sonicate for 15 s, centrifuge at 3000 g for 10 min, wash the precipitate with 2 mL water, centrifuge. Combine the aqueous layers and add 5 mL hexane, mix, centrifuge at 1500 g for 1 min, repeat the hexane wash. Evaporate the aqueous layer to dryness at low pressure, reconstitute with MeOH, centrifuge, evaporate the supernatant to dryness, reconstitute the residue with 3 mL water, inject a 50-500 μL aliquot on to column A and elute to waste with mobile phase A, after 3 min elute the contents of column A on to column B with mobile phase B and start the gradient, elute with mobile phase B and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 mm long 10 μm RP-18; B 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: A 100 mM Ammonium acetate buffer or 1% formic acid (?); B Gradient. A was 100 mM ammonium acetate buffer or 1% formic acid (?). B was MeCN:water 70:30 containing 100 mM ammonium acetate or 1% formic acid (?). A:B from 100:0 to 80:20 over 0.5 min, maintain at 80:20,for 1 min, to 25:75 over 10 min.
Flow rate: 1
Injection volume: 50-500
Detector: UV 254 or MS, Finnigan TSQ 70 triple quadrupole, Finnigan TSP source and interface, interface 80-85°, source 250°, manifold 70°, collision gas argon 0.4 mTorr, collision energy 40-50 eV

CHROMATOGRAM
Retention time: 12.1
Limit of detection: 400 pg (LC-SIM), 5-20 ng (MS-scan), 2 ng (UV)

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfanilamide, sulfapyridine, sulfathiazole
Interfering: sulfaquinoxaline (distinguish by MS)

KEY WORDS
cow; column-switching

REFERENCE
Abián,J.; Churchwell,M.I.; Korfmacher,W.A. High-performance liquid chromatography-thermospray mass spectrometry of ten sulfonamide antibiotics. Analysis in milk at the ppb level, *J.Chromatogr.*, **1993**, *629*, 267–276.

SAMPLE
Matrix: milk
Sample preparation: Sonicate (Branson Model 200 ultrasonic cell disruptor with a microtip probe) 50 mL milk for 2 min, cool to room temperature. Remove a 5 mL aliquot and add it to 25 mL dichloromethane:chloroform 75:25, invert 20 times, vortex horizontally at high speed for 15 s, centrifuge at 3300 g at 10° for 10 min. Remove 10 mL of the organic layer and evaporate it to just dryness under a stream of nitrogen at 32°, reconstitute the residue in 5 mL hexane, vortex for 30 s, add 1 mL 100 mM KH_2PO_4, shake briefly by hand, shake mechanically horizontally for 15 min, inject a 200 μL aliquot of the lower aqueous layer.

HPLC VARIABLES
Guard column: 50 mm long LC-18-DB (Supelco)
Column: 300 × 4.6 LC-18-DB (Supelco)
Mobile phase: MeOH:100 mM KH_2PO_4 40:60
Column temperature: 35
Flow rate: 1
Injection volume: 200
Detector: UV 269

CHROMATOGRAM
Retention time: 9.5
Limit of quantitation: 5 ppb

KEY WORDS
cow

REFERENCE
Weiss,G.; Laurencot,H.J.; MacDonald,A.; Duke,P.D.; Misra,K.; Horton,G.M.; Katz,S.E.; Brady,M.S. Determination of sulfadimethoxine withdrawal time from milk. Part I: Dosing, sampling and assay, *J.AOAC Int.*, **1995**, *78*, 358–371.

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 10.78

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfabenzamide, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:10 mM phosphate buffer 52:48
Flow rate: 1.5
Detector: F ex 400 em 500

OTHER SUBSTANCES
Simultaneous: sulfachlorpyridazine, sulfadoxine, sulfamethazine, sulfaquinoxaline, sulfathiazole

REFERENCE
Thomas,G.K.; Millar,R.G.; Anstis,P.W. Stability of sulfonamide antibiotics in spiked pig liver tissue during frozen storage, *J.AOAC Int.*, **1997**, *80*, 988–995.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 mm long MicroPak CN-10
Mobile phase: Isooctane:chloroform:MeOH:acetic acid 30.5:65:4:0.5
Flow rate: 0.33
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.99

OTHER SUBSTANCES
Simultaneous: sulfabromomethazine, sulfachlorpyridazine, sulfadiazine, sulfaethoxypridazine, sulfamethazine
Noninterfering: sulfamerazine, sulfathiazole, sulfanilamide, sulfapyridine, sulfaquinoline

REFERENCE
Seymour,D.; Rupe,B.D. High-pressure liquid chromatographic determination of sulfamethazine residues in beef tissues, *J.Pharm.Sci.*, **1980**, *69*, 701–703.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 25-40 μm LiChroprep Si 60 (Merck)
Column: 250 × 4 10 μm LiChrosorb Si 60
Mobile phase: Dichloromethane:MeOH:ammonia 80:19:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 289

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Simultaneous: N-acetylsulfadiazine, sulfadiazine, trimethoprim

KEY WORDS
normal phase

REFERENCE

Ascalone,V. Assay of trimethoprim, sulfadiazine and its N4-acetyl metabolite in biological fluids by normal-phase high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *224*, 59–66.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 39

OTHER SUBSTANCES

Simultaneous: sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 30:69:1
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 5.5

OTHER SUBSTANCES

Simultaneous: sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 1182–1185.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.56

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm Hypersil ODS RP-C18
Column: 100 × 4 5 μm Hypersil ODS RP-C18
Mobile phase: MeOH:50 mM pH 6.67 phosphate buffer 10:90
Flow rate: 0.8
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: sulfamethazine (sulfadimidine)

REFERENCE
van 't Klooster,G.A.E.; van Seeventer,P.B.; Kolker,H.J.; Smit,L.A.; Witkamp,R.F. High-performance liquid chromatographic method for the routine determination of sulphadimidine, its hydroxy metabolites and N4-acetylsulphadimidine in body fluids and cell culture media, *J.Chromatogr.*, **1991**, *571*, 157–168.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 189.5

OTHER SUBSTANCES
Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadoxine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine, sulfisoxazole

REFERENCE
Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, gbuprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 0.35 5 μm Vydac *IDI*-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)

Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 53

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 61

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: tissue
Sample preparation: 500 mg Tissue + 2 g C18 material + 5 μL MeOH + 5 μL 80 μg/mL sulfamethoxazole in MeOH, let stand for 2 min, grind gently with a glass pestle until homogeneous, place in a 10 mL syringe barrel plugged with filter paper, place filter paper on top, compress to 4.5 mL with a plunger, restrict column outlet with a 100 μL pipette tip, wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL dichloromethane,

elute excess dichloromethane with positive pressure. Evaporate the eluate under a stream of nitrogen at 40°, dissolve the residue in 500 μL mobile phase, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter supernatant (0.45 μm), inject a 25 μL aliquot. (C18 material was Analytichem 40 μm 18% load endcapped. Add 22 g to a 50 mL syringe barrel wash with 2 column volumes of hexane, 2 volumes of dichloromethane, and 2 volumes of MeOH, vacuum aspirate until dry.)

HPLC VARIABLES
Column: 300 × 4 MicroPak ODS
Mobile phase: MeCN:17 mM orthophosphoric acid 35:65
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 270

CHROMATOGRAM
Retention time: 8
Internal standard: sulfamethoxazole (6)
Limit of detection: 1.25 ng

KEY WORDS
muscle; fish; catfish; matrix solid phase dispersion

REFERENCE
Long,A.R.; Hsieh,L.C.; Malbrough,M.S.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of sulfadimethoxine in catfish (Ictalurus punctatus) muscle tissue, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 868–871.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 5 g Fish + 1 mL 100 μg/mL carbamazepine diol in MeOH + 15 mL MeCN + 500 μL 50% trichloroacetic acid, homogenize (Brinkmann Polytron PT 10/35) at medium speed for 30 s, centrifuge at 4° at 7800 g for 25 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 5 mL water, vortex for 30 s, filter (13 mm dia. 8 μm Membra-Fil (Nucleopore)), add the filtrate to the SPE cartridge, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 20 μL aliquot. (Flush injection valve with 1 mL mobile phase between analyses.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18
Column: 250 × 4.6 5 μm Ultrasphere
Mobile phase: MeCN:MeOH:100 mM pH 4.0 phosphate buffer 17:10:73
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 27.5
Internal standard: carbamazepine diol (10.5)
Limit of quantitation: 0.2 ppm

OTHER SUBSTANCES
Extracted: ormetoprim
Simultaneous: sulfacetamide, sulfadiazine, sulfamerazine, sulfamethazine, sulfisoxazole

KEY WORDS
fish; salmon; SPE; pharmacokinetics

REFERENCE
Walisser,J.A.; Burt,H.M.; Valg,T.A.; Kitts,D.D.; McErlane,K.M. High-performance liquid chromatographic analysis of Romet-30 in salmon following administration of medicated feed, *J.Chromatogr.*, **1990**, *518*, 179–188.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL water. Homogenize 5 g tissue with 100 mL MeOH:0.2% metaphosphoric acid 40:60 for 2 min, filter through a 1 mm layer of Hyflo Super-Cel. Evaporate the filtrate under reduced pressure at 40° to 10 mL, add the residue to the SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, take up the residue in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Newguard RP-8
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:5 mM oxalic acid 45:55
Flow rate: 0.5
Injection volume: 10
Detector: UV 265

CHROMATOGRAM

Retention time: 8
Limit of detection: 50 ng/g

OTHER SUBSTANCES

Extracted: sulfamonomethoxine, sulfisozole, nalidixic acid, oxolinic acid, piromidic acid, sodium nifurstyrenate, furazolidone

KEY WORDS

fish; SPE

REFERENCE

Horie,M.; Saito,K.; Hoshino,Y.; Nose,N.; Nakazawa,H.; Yamane,Y. Simultaneous determination of residual synthetic antibacterials in fish by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *538*, 484–491.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allow to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 μL initial mobile phase, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 0.8

Injection volume: 50

Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 μL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 28
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Brinkmann Polytron) 40 g salmon tissue and 200 mL acetone for 3 min, add 10 g Celite, add 20 g sodium sulfate, blend for 2 min, filter, wash the solid with three 15 mL portions of acetone. Evaporate the filtrate to dryness under educed pressure at 40°, reconstitute with 100 mL dichloromethane. Remove a 50 mL aliquot and add it to 100 mL 100 mM NaOH, shake vigorously, centrifuge at 10° at 12 g for 20 min. Remove the aqueous layer and neutralize it with HCl, freeze dry overnight, remove the remaining water under reduced pressure at 40°, reconstitute the remaining residue with 5 mL MeOH:water 25:75, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC 18DB
Mobile phase: MeCN:water 35:65 containing 0.1% formic acid
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 7.3
Limit of detection: 13 ng/g

OTHER SUBSTANCES
Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS
salmon; fish

REFERENCE
Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE
Matrix: tissue
Sample preparation: Blend 3 g meat with 30 mL chloroform for 2 min in a Polytron homogenizer, shake for 10 min, centrifuge at 1600 g for 5 min, filter (5A filter paper). Add 5 mL filtrate to 1 mL 3 M HCl, shake for 10 min, centrifuge at 1600 g for 5 min. 250 μL Aqueous layer + 250 μL 3.5 M sodium acetate solution, vortex, add 100 μL 0.2% fluorescamine in acetone, vortex, let stand for 20 min at room temperature, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:2% acetic acid 5:3
Column temperature: 55
Flow rate: 1
Injection volume: 10
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 14.5
Limit of detection: 0.005 ng/g

OTHER SUBSTANCES
Simultaneous: sulfisomidine, sulfamethoxazole, sulfamerazine, sulfadiazine, sulfamonomethoxine, sulfamethazine (sulfadimidine), sulfaquinoxaline

KEY WORDS
cow; pig; chicken; ham; sausage; roast beef; derivatization

REFERENCE
Takeda,N.; Akiyama,Y. Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products, *J.Chromatogr.*, **1991**, *558*, 175–180.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g ground tissue with 40 mL acetone, centrifuge at 2800 g for 5 min, filter (paper) the supernatant. Homogenize (Polytron) the residue with 20 mL acetone for 1 min, centrifuge, filter. Combine the filtrates and add 60 mL 125 mM HCl, wash twice with 50 mL portions of n-hexane, add 10 mL 1 M pH 5.2 acetate buffer, adjust pH to 5.0-5.1 with 5 M NaOH, extract with 60 ml and 40 mL portions of ethyl acetate, combine the organic layers, evaporate to about 2 mL under reduced pressure at 45°C, add about 15 mL EtOH, evaporate to dryness under reduced pressure at 50°, reconstitute immediately with 5-7 mL dichloromethane. Add to an 85 mm long column of silica gel made up in dichloromethane, rinse the flask twice with 1-2 mL portions of dichloromethane, add the rinses to the column, elute with 40 mL acetone:dichloromethane (60:40), elute to waste until the acetone front (visible against a dark background) is about 10 mm from the end of the column, collect the remaining eluate (Mitt. Gebiete. Lebensm. Hyg. 1990, 81, 522). Add 150 μL 10 μg/mL sulfabenzamide to the eluate, evaporate to dryness under reduced pressure at 45°, reconstitute the residue in 300 μL MeOH:water 50:50, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 15.5
Internal standard: sulfabenzamide (8.8)
Limit of detection: 2 ppb

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
post-column reaction; muscle; liver; kidney; SPE

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 μL 20 μg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min. Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72
Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM
Retention time: 23.0
Internal standard: sulfaethoxypyridazine (12.8)
Limit of detection: 10 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazole

KEY WORDS
cow; pig; muscle; liver; SPE

REFERENCE
Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 3 g ground tissue with 30 mL chloroform for 2 min, centrifuge at 3000 g for 5 min, filter (paper). Remove a 10 mL aliquot of the filtrate and add it to 1 mL 3 M HCl, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove a 250 μL aliquot of the aqueous layer and add it to 250 μL 3.8 M sodium acetate, add 100 μL 1 mg/mL fluorescamine in MeCN, vortex, let stand at room temperature for 20 min, inject a 20 μL aliquot.)Sodium acetate should be a highly pure grade.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 120 C18

Mobile phase: MeCN:20 mM pH 4 NaH_2PO_4 34:66 containing 20 mM sodium octanesulfonate
Column temperature: 30
Flow rate: 1.2
Injection volume: 20
Detector: F ex 405 em 495

CHROMATOGRAM
Retention time: 21
Limit of detection: 9 ng/g

OTHER SUBSTANCES
Extracted: sulfadiazine, sulfamethazine, sulfaquinoxaline

KEY WORDS
derivatization; chicken; muscle

REFERENCE
Simeonidou,E.J.; Botsoglou,N.A.; Psomas,I.E.; Fletouris,D.J. Liquid chromatographic analysis of multiple sulfonamide residues in chicken muscle using pre-column derivatization and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2349–2364.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

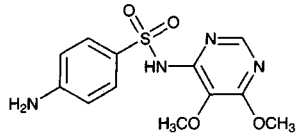
CHROMATOGRAM
Retention time: 23.5
Limit of quantitation: 1 ng/g

OTHER SUBSTANCES
Extracted: sulfachloropyridazine, sulfadiazine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

Sulfadoxine



Molecular formula: $C_{12}H_{14}N_4O_4S$
Molecular weight: 310.33
CAS Registry No.: 2447-57-6
Merck Index: 9074
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 3 mL MeOH and 3 mL 50 mM pH 3.4 oxalate buffer. 1 mL Plasma + 1 mL 50 mM pH 3.4 oxalate buffer + 50 µL 200 µg/mL IS in MeOH:water 50:50, mix, add to the SPE cartridge. Wash with 3 mL 50 mM pH 3.4 oxalate buffer, 1 mL MeOH:water 20:80, and 2 mL hexane:ether 80:20. Elute with two 1 mL portions of MeOH:25% ammonia solution 99:1. Evaporate the eluate to dryness under a gentle stream of nitrogen at 30°. Dissolve the residue in 250 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.9 5 µm Symmetry C18 (Waters)
Column: 250 × 4.6 5 µm Symmetry C18 (Waters)
Mobile phase: MeCN:MeOH:water 25:10:65 containing 1% triethylamine, adjusted to pH 5.6 with phosphoric acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Internal standard: sulfadimethoxine
Limit of detection: 14.61 ng/mL
Limit of quantitation: 22.08 ng/mL

OTHER SUBSTANCES

Extracted: pyrimethamine
Simultaneous: acetaminophen, 4-chlorophenylbiguanide, cycloguanil, proguanil, quinine, sulfadiazine,

KEY WORDS

plasma; SPE

REFERENCE

Astier,H.; Renard,C.; Cheminel,V.; Soares,O.; Mounier,C.; Peyron,F.; Chaulet,J.F. Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction, *J.Chromatogr.B*, **1997**, *698*, 217–223.

SAMPLE

Matrix: blood
Sample preparation: Add 150 µL 100 mM zinc sulfate to 600 µL plasma while vortexing over 15 s, add 700 µL MeCN containing 4 µm WR 184806 and 75 µM sulfadimethoxine while vortexing over 15 s, , let stand for 15 min, centrifuge at 10000 g for 10 min. Remove the supernatant and add it to 2 mL pH 9.0 phosphate buffer, add 2 mL 60 mM tetrabutylammonium hydroxide, add 5 mL MTBE, shake for 10 min, centrifuge at 1200 g for 5 min. Remove the upper organic layer and evaporate it to dryness at 50°, reconstitute the residue in 200 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 150 × 4 3 µm Spherisorb S3-ODS-1
Mobile phase: MeCN:100 mM phosphate buffer 48:52, adjusted to pH 3.5

Flow rate: 0.5
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 5.31
Internal standard: sulfadimethoxine (6.22), 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-tert-butylamino)propyl]quinoline phosphate (WR 184806) (Walter Reed) (21.00)
Limit of detection: 75 μM

OTHER SUBSTANCES
Extracted: mefloquine, pyrimethamine

KEY WORDS
plasma

REFERENCE
Bergqvist,Y.; Eckerbom,S.; Larsson,H.; Malekzadeh,M. Reversed-phase liquid chromatographic method for the simultaneous determination of the antimalarial drugs sulfadoxine, pyrimethamine, mefloquine and its major carboxylic metabolite in plasma, *J.Chromatogr.*, **1991**, *571*, 169–177.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma, whole blood, or red blood cells + 100 μL 50 μg/mL sulfamethoxazole in MeOH + 500 μL water + 100 μL buffer + 6 mL ethylene dichloride, shake on an orbital mixer for 20 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness at 60° on a vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. (Buffer was prepared by adding 100 μL acetic acid to 9.9 mL phosphate buffer, pH 3.40.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:1 M perchloric acid:water 30:9:0.8:95
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: sulfamethoxazole (8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, dapsone, primaquine, pyrimethamine, quinidine, quinine, sulfalene

---

KEY WORDS
plasma; whole blood; red blood cells; pharmacokinetics

REFERENCE
Dua,V.K.; Sarin,R.; Sharma,V.P. Sulphadoxine concentrations in plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases after treatment with Fansidar using high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1317–1323.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Whole blood + 100 μL 200 mM zinc sulfate + 200 μL MeCN + sulfadimethoxine, vortex, centrifuge at 6000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL C18

Mobile phase: MeCN:MeOH:17 mM orthophosphoric acid 5:10:85
Column temperature: 45
Flow rate: 1.5
Detector: UV 270

CHROMATOGRAM
Internal standard: sulfadimethoxine

OTHER SUBSTANCES
Noninterfering: sulfalene, sulfamethoxazole

KEY WORDS
whole blood; comparison with colorimetric procedure

REFERENCE
Green,M.D.; Mount,D.L.; Todd,G.D. Determination of sulfadoxine concentrations in whole blood using C18 solid-phase extraction, sodium dodecyl sulfate and dimethylaminocinnamaldehyde, *Analyst*, **1995**, *120*, 2623–2626.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.345

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column

A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)

Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5

Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 2.6

Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole

Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: eggs, milk, tissue

Sample preparation: Milk. Centrifuge at 2000 g and freeze at -20° to remove the cream. Mix a 5 mL aliquot with 5 mL saline solution and add 1 mL 1% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Meat. Blend 10 g homogenized meat with 20 mL saline, centrifuge, remove a 10 mL aliquot of the clear upper phase and add it to 1 mL 1% sodium azide (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Eggs. Dilute 10 g homogenized whole egg with 10 mL saline, add 3 mL 10% sodium azide solution (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!). Dialyze (24" long

cellulose acetate "Type C" membrane, Technicon, New York) an 8 mL aliquot against water pumped at 0.6 mL/min, pass the dialysate through column A to waste, after 10 min stop the dialysis and elute column A to waste with water, after 24 min backflush the contents of column A onto column B with mobile phase, after 5 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 60 × 4 50-100 μm XAD-4 (Rohm & Haas); B 250 × 4.6 7 μm Cp TM-Spher C18 (Chrompack)
Mobile phase: MeCN:50 mM pH 6.85 sodium acetate buffer 12.5:87.5
Detector: UV 450 following post-column reaction. The column effluent mixed with 1.5% p-dimethylaminobenzaldehyde in 17% phosphoric acid and the mixture flowed through a 7.5 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Retention time: k' 2.6
Limit of detection: 5-10 ng/g

OTHER SUBSTANCES

Extracted: dapsone, sulfacetamide, sulfachlorpyrazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfaquinoxaline, sulfathiazole, sulfatroxazole
Noninterfering: chloramphenicol, trimethoprim

KEY WORDS

post-column reaction; meat; column-switching; dialysis

REFERENCE

Aerts,M.M.L.; Beek,W.M.J.; Brinkman,U.A.T. Monitoring of veterinary drug residues by a combination of continuous flow techniques and column-switching high-performance liquid chromatography. I. Sulphonamides in egg, meat and milk using post-column derivatization with dimethylaminobenzaldehyde, *J.Chromatogr.*, **1988**, *435*, 97–112.

SAMPLE

Matrix: milk
Sample preparation: Mix 5 mL milk with 20 μL 12 M HCl, sonicate, add 25 mL ethyl acetate, extract using a rotary shaker (REAX 2, Heidolph) for 10 min. Centrifuge at 1500 g for 5 min, evaporate 20 mL of the ethyl acetate extract to dryness, dissolve the residue in 10 mL 1 HCl. Wash the aqueous phase with 10 mL hexane, adjust to pH 5.5 with 900 μL 10 M NaOH and 5 mL 1 M pH 6.0 KH_2PO_4, extract with two 10 mL portions of dichloromethane. Evaporate the organic layer to dryness, dissolve the residue in 2 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Novapak C18 (Waters)
Mobile phase: MeCN:10 mM pH 6.6 ammonium acetate 10:90
Flow rate: 1
Injection volume: 50
Detector: UV 271

CHROMATOGRAM

Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Also analyzed: sulfadimethoxine, sulfadimidine (UV 265), sulfamethoxypyridazine (UV 265)

KEY WORDS

cow; milk

REFERENCE

Roudaut,B.; Moretain,J.P. Sulphonamide residues in milk of dairy cows following intravenous injection, *Food Addit.Contam.*, **1990**, *7*, 527–533.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:10 mM phosphate buffer 52:48
Flow rate: 1.5
Detector: F ex 400 em 500

OTHER SUBSTANCES
Simultaneous: sulfachlorpyridazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline, sulfathiazole

REFERENCE
Thomas,G.K.; Millar,R.G.; Anstis,P.W. Stability of sulfonamide antibiotics in spiked pig liver tissue during frozen storage, *J.AOAC Int.*, **1997**, *80*, 988–995.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax-Sil
Mobile phase: Dichloromethane:MeOH:1 M perchloric acid 100:9:0.4
Flow rate: 0.8
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 7.4

OTHER SUBSTANCES
Simultaneous: amodiaquine, chloroquine, dapsone, desethylchloroquine, dihydroquinidine, dihydroquinine, primaquine, proguanil, pyrimethamine, quinidine, quinine, sulfalene, sulfamethoxazole
Interfering: mefloquine

KEY WORDS
normal phase

REFERENCE
Dua,V.K.; Sarin,R.; Prakash,A. Determination of quinine in serum, plasma, red blood cells and whole blood in healthy and *Plasmodium falciparum* malaria cases by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 87–93.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:10 mM pH 5.6 phosphate buffer 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 56.0

OTHER SUBSTANCES
Simultaneous: N-acetylsulfisomidine, sulfachloropyridazine, sulfadimethoxine, sulfamethazine (sulfadimidine), sulfamethoxypyridazine, sulfamonomethoxine, sulfisomidine, sulfisoxazole

REFERENCE
Nishikawa,M.; Takahashi,Y.; Ishihara,Y. High performance liquid chromatographic determination of sulfisomidine and N4-acetylsulisomidine in swine tissues, *J.Liq.Chromatogr.*, **1993**, *16*, 4031–4047.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Chromabond SA cation-exchange SPE cartridge (Macherey-Nagel) with 6 mL hexane, dry under vacuum for 10 min, condition with 6 mL dichloromethane:acetone:acetic acid 50:50:2, do not allot to go dry. Homogenize (Polytron) 10 g sample with 60 mL dichloromethane:acetone 50:50 for 30 s, rinse the apparatus with 2-3 mL dichloromethane:acetone 50:50, centrifuge the mixture at 2500 rpm for 10 min. Filter (cotton wool) the supernatant and wash it through with a little dichloromethane:acetone 50:50, add 5 mL acetic acid to the filtrate, mix, remove one tenth of this mixture and add it to the SPE cartridge at 2 mL/min, do not allow the SPE cartridge to run dry, wash with 5 mL water, wash with 5 mL MeOH, dry under vacuum for 10 min, pass gaseous ammonia through the SPE cartridge until the acid is neutralized (when air is passed through the cartridge moist pH paper should turn blue), elute with 3 mL MeOH at 1-2 mL/min, carefully evaporate to dryness under reduced pressure (100 mbar) at 40°, reconstitute with 500 µL initial mobile phase, centrifuge, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 µm LiChrospher 100 RP-18
Mobile phase: Gradient. A was MeCN:20 mM pH 5 sodium acetate buffer 5.5:94.5. B was MeCN:EtOH:20 mM pH 5 sodium acetate buffer 50:10:40. A:B from 100:0 to 0:100 over 32 min (concave gradient), return to initial conditions over 4 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 270, F ex 395 em 495 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.3 mL/min and this mixture flowed through a 2.3 m × 0.5 mm ID coil in a cooled ultrasonic bath to the detector. (Prepare reagent by dissolving 25 mg fluorescamine in 25 mL MeCN and adding 75 mL buffer and 200 µL mercaptoethanol. Buffer was 20 mM NaH_2PO_4 adjusted to pH 3 with 1 M phosphoric acid.)

CHROMATOGRAM
Retention time: 24
Limit of detection: 0.5-5 ppb

OTHER SUBSTANCES
Extracted: sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfamethazine (sulfadimidine), sulfamethizole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Pacciarelli,B.; Reber,S.; Douglas,C.; Dietrich,S.; Etter,R. Determination of 12 sulfonamides in meat and kidney by HPLC with post-column derivatization, *Mitt.geb.Lebensmittelunters.Hyg.*, **1991**, *82*, 45–55.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Sep-Pak SPE cartridge with 20 mL MeOH and 20 mL water. 5 g Homogenized tissue + 40 µL 20 µg/mL sulfaethoxypyridazine in water + 25 mL chloroform, shake mechanically for 2 min, centrifuge at 3000 g for 5 min, remove the supernatant and separate the layers. Add the aqueous layer to the residue and repeat the extraction. Combine the chloroform layers and add 10 mL 10% NaCl in 100 mM NaOH, shake vigorously for 1 min, remove the upper aqueous layer and centrifuge it at 1500 g for 10 min.

Remove 8 mL of the upper aqueous layer and add it to 10 mL 1 M pH 6 NaH_2PO_4, vortex for 20 s, add to the SPE cartridge, wash with 20 mL water, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute in 2 mL mobile phase, vortex for 20 s, heat at 50° for 5 min, cool, filter (Gelman Acrodisc 0.45 μm), inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeCN:10 mM pH 4.6 ammonium acetate 28:72
Flow rate: 1.2
Injection volume: 20-50
Detector: UV 265 or MS, VG TRIO 2 quadrupole, ion source 189°, capillary jet 320

CHROMATOGRAM

Retention time: 10.4
Internal standard: sulfaethoxypyridazine (12.8)

OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfathiazole

KEY WORDS

cow; pig; muscle; liver; SPE

REFERENCE

Boison,J.O.; Keng,L.J.-Y. Determination of sulfadimethoxine and sulfamethazine residues in animal tissues by liquid chromatography and thermospray mass spectrometry, *J.AOAC Int.*, **1995**, *78*, 651–658.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T-25 with S25N dispersing tool) 10 g chopped fish and 10 mL mobile phase A at high speed for 30 s, add 90 mL MeCN, shake at low speed on shaker, centrifuge at 1500 rpm for 10 min, remove the supernatant, add 30 mL MeCN to the solid, shake, centrifuge, decant the supernatant. Combine the supernatants, add 100 mL water, add 2 mL diethylene glycol, add 60 mL dichloromethane, shake for 3 min, remove the organic layer, repeat the extraction with 40 mL dichloromethane. Combine the organic layers and evaporate in a rotary evaporator at 65° to ca. 2 mL, wash into a smaller tube with two 2 mL portions of MeOH, concentrate to about 1 mL with a stream of nitrogen at 65°, dilute to 4.5 mL with 200 mM phosphoric acid, add 5 mL hexane, vortex, centrifuge for 15 min, discard upper hexane layer. Dilute the lower aqueous layer to 5 mL with 200 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:MeOH:2% acetic acid in water 5:10:85. B was MeCN:MeOH:2% acetic acid in water 25:10:65. A:B from 100:0 to 0:100 over 25 min, maintain at 0:100 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 400 em 495 following post-column reaction. The column effluent mixed with 500 μg/mL fluorescamine in MeCN:MeOH:2% acetic acid 52.5:5:42.5 pumped at 0.2 mL/min and the mixture flowed through a 10.7 m × 0.4 mm i.d. coil of PTFE tubing at 70° to the detector.

CHROMATOGRAM

Retention time: 15
Limit of quantitation: 1 ng/g

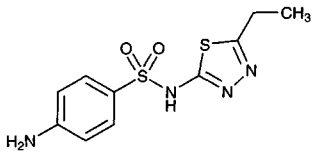
OTHER SUBSTANCES

Extracted: sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole

KEY WORDS
fish; salmon; post-column reaction

REFERENCE
Gehring,T.A.; Rushing,L.G.; Thompson,H.C.,Jr. Determination of sulfonamides in edible salmon tissue by liquid chromatography with postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1997**, *80*, 751–755.

Sulfaethidole



Molecular formula: $C_{10}H_{12}N_4O_2S_2$
Molecular weight: 284.36
CAS Registry No.: 94-19-9
Merck Index: 9075
Lednicer No.: 1 125

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

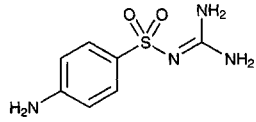
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Sulfaguanidine



Molecular formula: $C_7H_{10}N_4O_2S$
Molecular weight: 214.25
CAS Registry No.: 57-67-0
Merck Index: 9076
Lednicer No.: 1 123

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

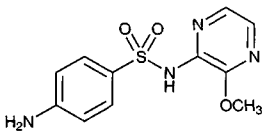
Retention time: 3.795

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sulfalene



Molecular formula: $C_{11}H_{12}N_4O_3S$
Molecular weight: 280.31
CAS Registry No.: 152-47-6, 50933-06-7 (mixture with trimethoprim)
Merck Index: 9078
Lednicer No.: 1 125

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

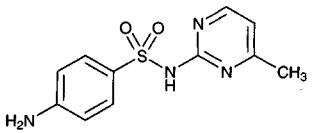
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.217

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Sulfamerazine



Molecular formula: $C_{11}H_{12}N_4O_2S$
Molecular weight: 264.31
CAS Registry No.: 127-79-7, 127-58-2 (monosodium salt)
Merck Index: 9081
Lednicer No.: 1 124

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: water

Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 5 mL 1 mg/mL niacin in 0.5 mM HCl, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 6
Internal standard: niacin (3.3)

OTHER SUBSTANCES

Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamethoxazole, sulfamonomethoxine

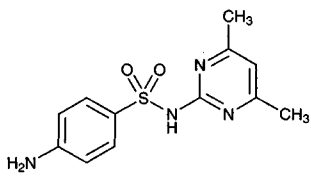
KEY WORDS

wastewater

REFERENCE

Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Sulfamethazine



Molecular formula: $C_{12}H_{14}N_4O_2S$
Molecular weight: 278.33
CAS Registry No.: 57-68-1
Merck Index: 9083
Lednicer No.: 1 125

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 150 μL 3% trichloroacetic acid in EtOH + 100 μL EtOH, vortex, freeze at -20° for 5 min, centrifuge, freeze at -20° for 10 min, centrifuge through a Spin-X filter tube, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18 DB
Column: 250 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:buffer 23:77 with 0.1% triethylamine added (Buffer was 25 mM sodium phosphate and 20 mM sodium 1-hexanesulfonate, pH adjusted to 2.8 with 5 M phosphoric acid.)
Flow rate: 0.9
Injection volume: 10
Detector: UV 270

CHROMATOGRAM

Retention time: 8
Internal standard: sulfamethazine (sulfadimidine)